DNA extraction protocol for PST spores

If using non-germinated spores

- Add 250 microliter of extraction buffer to the mortar,
- Then add the spores and grind with force (very quickly),
- Recover this with a pipet and transfer to a new tube,
- then add remaining 250 microliters of extraction buffer

1. Grind 100 mg of spores.
2. Add 500 microliters of extraction buffer (listed below)
3. Add 500 microliters of phenol:chloroform:Isoamyl Alcohol (25:24:1) pH7.5
4. Mix for 10 minutes in a vertical shaker (250 rpm)
5. Centrifuge for 15 minutes at 10,000 rpm
6. Transfer supernatant to a fresh tube
7. Add 500 microliters Chloroform: Isoamyl Alcohol (24:1)
8. Mix for 10 minutes in a vertical shaker (250 rpm)
9. Centrifuge for 10 minutes at 13,000 rpm
10. Transfer supernatant to a new tube
11. Add 1 volume Isopropanol. Mix slowly.
12. Centrifuge 13,000 rpm for 2 minutes. Discard supernatant
13. Wash pellet with ethanol 70%
14. Centrifuge 13,000 rpm for 2 minutes. Discard supernatant
15. Let dry for about 10 minutes
16. Re-suspend in 30 microliters of elution buffer (EB)
17. Add 1 microliter of RNAse for 1 hour at 37°C.
18. Quantify and freeze at -20°C

Extraction Buffer

- 1.5 g Glycine-NaOH pH 9.0
- 2 ml 5M NaCl
- 4 ml 0.5M EDTA, pH 8.0
- 20 ml 20% SDS
- 2 g Sodium Lauryl Sarcosine

In 100 ml of ddH2O add 1.5g glycine and 2 ml 5M NaCl, take that to pH 9.0 using NaOH (it may take some time). Add the remaining ingredients and dissolve up to 200 ml with ddH2O

Points to consider

- It is **highly recommended** to isolate DNA from one isolate at the time, to avoid accidental cross contamination from one SPI to another, especially when grinding.
- For DNA quantification, be careful with values from instruments such as the NanoDrop. We've had very bad experience with the results. Ideally, samples should be measured with Qbit **and** run on an agarose gel.
- To sequence with our genome center, we require a minimum of 5ug of DNA in at least 25ng/ul concentration.